COLD, NOT WARM TEMPERATURES INFLUENCE ONSET OF INCUBATION AND HATCHING FAILURE IN HOUSE WRENS (*TROGLODYTES AEDON*)

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Taza Dawn Schaming

May 2010

ABSTRACT

Understanding the patterns and mechanisms underlying expression of avian life history traits, including seasonal variation in the onset of incubation and clutch size, will shed light on the plasticity of avian responses to environmental change generally, and global climate change in particular. It is commonly believed that hyperthermia is more injurious to the developing embryo than hypothermia. However, most studies evaluate effects of high but not low temperatures on egg viability, and do not examine how exposure to both warm and cold temperatures influence individual laying and incubation behavior. In my study, I evaluated the behavioral responses, specifically onset of incubation and clutch size determination, of female House Wrens, *Troglodytes aedon*, to warm and cold ambient temperatures. I then assessed hatching failure as a function of egg exposure to these high and low temperatures.

To quantify within species variation in onset, I used a continuous record of incubation from clutch initiation through hatching. Females were not significantly more likely to initiate incubation prior to clutch completion with increased duration of pre-incubation temperatures above physiological zero ($\geq 24°C$); however, the probability of a female initiating incubation early decreased with longer exposure to cold ($\leq 16°C$) weather. Females did not tend to initiate incubation early with larger clutches, and clutch size did not decrease or increase significantly with increasing exposure to warm ($\geq 24°C$) or cold ($\leq 16°C$) pre-incubation temperatures, respectively. The likelihood of partial hatching failure within a clutch and the per-egg probability of not hatching did not decrease with increased exposure to warm pre-incubation temperatures, but did increase with the time pre-incubation temperatures were hypothermic ($\leq 16°C$). Partial hatching failure was not significantly higher in larger clutches, and was not more likely in earlier laid eggs. These results suggest that,

contrary to common belief, in temperate climates, egg exposure to colder rather than warmer temperatures may be a more influential factor affecting decreases in egg viability: in House Wrens in Ithaca, New York, hatchability declined with exposure to cold, but not warm temperatures. In cold weather, females were likely constrained by the environmental conditions, and due to an increased need to spend more energy on self-maintenance, did not initiate incubation early enough to counteract the negative effects of cold temperature on egg viability.

BIOGRAPHICAL SKETCH

Taza Schaming was born in Albany, New York, U.S.A. on September 4th, 1979. She grew up on a small farm, spending the majority of her time outside in the thousands of hectares of woods that extended beyond her backyard, learning tracking, bird identification and a number of outdoor survival skills. She knew at a very young age that she wanted to grow up to be a scientist, working in the field, figuring out how the world worked. Taza studied biology at Tufts University, and received a Bachelor of Science in 2001. While in school, Taza attended the School for International Training's biodiversity and conservation program in Tanzania, studying tree dominance in the montane rainforest of Mazumbai, worked in Dr. Meller's lab, studying gene expression in *Drosophila melanogaster*, and assisted in a graduate project, assessing terrestrial habitat requirements of *Ambystomatidae laterale*, blue-spotted salamanders, a species of special concern. Taza then lived, worked, and traveled around the world for six years before beginning graduate school at Cornell University in 2007. She worked for Point Reyes Bird Observatory in the sagebrush habitat of southeastern Wyoming, investigating the effects of natural gas development on avian survival and reproductive success, volunteered with Inti Wara Yassi, an animal rehabilitation center in Villa Tunari, Bolivia, worked for the U.S. Fish and Wildlife Department in eastern Massachusetts, then as a field technician for Environmental Compliance Services, a private consulting company. While at ECS, she enrolled in her first graduate classes, Environmental Management I and II, at Harvard University. Taza has also spent three seasons backcountry snowboarding in the Rockies and Andes, learning difficult orienteering skills, and extreme self-reliance and independence. She spent a year in South America, climbing and snowboarding in remote mountains, volunteering, and exploring the diverse cultures and ecosystems of

the Andes. She has also studied in Australia, trekked in Nepal, and travelled through Kenya, Malaysia and New Zealand. Taza plans to spend her life working in remote, high alpine ecosystems around the world, studying ornithology and ecology, dedicating herself to better understanding and conserving these wild lands.

I dedicate this thesis to my mother and father, Teal and Peter Schaming, for encouraging me to never to stop asking questions and to never stop learning.

ACKNOWLEDGMENTS

I would like to thank Janis Dickinson, my advisor, for being my mentor, for asking me difficult questions, and for allowing me to be independent while still providing constant support and feedback. I owe much gratitude to Becky Cramer for her constant good humor, and for being such a pleasure to work with so closely on this project, Paulo Llambías for inspiring me with his passion for talking about both birds and mountains, and his advice on this research every step of the way, and to Benjamin Zuckerberg for many, many hours of patient statistical help and advice. I would like to thank my committee member André Dhondt, for providing such excellent advice and for sharing his experience, Rebecca Lohnes, for being a sounding board for all of my ideas, and for helping guide me through the process of graduate school, and Caren Cooper for her inspiration in my deciding on this project. I would also like to thank Katie LaBarbera and Katherine Anguish for help in the field, Pat Sullivan for statistical help, Don Lisk for volunteering to build nest boxes, and David Bonter, James Goetz, Caitlin Stern, Andrea Townsend, and Margaret Voss for advice and comments. I thank my good friend, Laura Martin for her wonderful advice, and for being a role model with her incredible work ethic, and Mia Davis for her constant support and friendship. I thank the faculty, graduate students, and staff of the Cornell Lab of Ornithology and the Department of Natural Resources for all of their help, advice and encouragement along the way. Lastly I would like to thank my parents, Teal and Peter Schaming, for being such wonderful role models in work and life, for constantly discussing my work and giving me new ideas, and supporting and encouraging me every step of the way, and my sister, Jada, for always making me feel like I can do anything.

This work was completed with the support of the Cornell University College of Agriculture and Life Sciences Science Fellowship, Cornell University Lab of Ornithology, Cornell University Department of Natural Resources, and National Sigma Xi Grants-In-Aid-of-Research. Data collection was carried out under U.S. Fish and Wildlife Service permit #23533, issued to J.L.D. with T.D.S. as authorized subpermittee, Cornell University Institutional Animal Care and Use Committee protocol #2008-0048, and New York scientific collecting permits #1256, issued to T.D.S. by the New York State Department of Fish and Wildlife.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Several climate models predict increased variability in temperature in the near future, with a rise in mean temperatures for North America (Pendlebury et al. 2004). Climate change has already influenced a diversity of life history traits, including plant flowering phenology (Parmesan and Yohe 2003), butterfly migration dates (Peñuelas et al. 2002), mosquito growing season length (Bradshaw and Holzapfel 2001), and range shifts for trees (Walther et al. 2005), insects (Hill et al. 1999) and mammals (Humphries et al. 2002). It has also influenced several avian life history traits, such as lay date, clutch size, and migration patterns (Ahola et al. 2004, 2009). In birds, onset of incubation, clutch size, and hatching failure are considered to be linked and sensitive to environmental temperatures (Cooper et al. 2005). As both warm and cold ambient temperatures potentially influence egg viability, a general theory should consider and test the importance of both extremes to understand the nature and flexibility of life history adaptations to changing climate.

Birds may respond to high and low temperatures by initiating incubation prior to the day the last egg is laid, or by altering their clutch size. In the process, they may balance their investment and behavior to counteract negative effects of temperature on egg viability. By assessing whether avian species alter their laying and incubation behavior with temperature, and the degree to which birds are constrained by environmental conditions, we can better predict the resilience of bird populations to global climate change. A better understanding of individual responses to local environmental conditions may also help to explain large-scale geographic and seasonal trends, such as smaller clutch sizes later in the season and at lower latitudes (Cooper et al. 2005, 2006).

Onset of incubation in birds is variable within and among species, with some initiating incubation as early as the day the first egg is laid, and others beginning only when the clutch is complete (Clark and Wilson 1981). Females initiating incubation before clutch completion is puzzling, because there are multiple potential costs for incubators. These costs include a decrease in time available for self-maintenance due to direct tradeoffs between incubation and foraging (Slagsvold and Lifjeld 1989), and potential costs of asynchronous hatching, which may increase competition among chicks within the nest, leading to brood reduction or, in extreme cases, siblicide (Stoleson and Beissinger 1995, Viñuela 1999).

The egg-viability (Veiga 1992, Stoleson and Beissinger 1999) and egg-hypothermia hypotheses attempt to explain variation in the onset of incubation as a function of temperature; females initiate incubation before clutch completion ("early onset") to decrease egg mortality resulting from exposure to unfavorably warm or cold temperatures. Together, these hypotheses propose that avian laying and incubation behavior involves a complex strategy, which balances the potentially detrimental effects of warm as well as cold temperature extremes.

The egg-viability hypothesis proposes that females exposed to higher ambient temperatures will initiate incubation before clutch completion to increase egg survival (Stoleson and Beissinger 1999). In other words, ambient temperatures above physiological zero trigger onset of embryonic development, and developing embryos are more sensitive to warming than are undeveloped eggs; females can best protect eggs from mortality due to temperature exposure by beginning to incubate before the last egg is laid (Stoleson and Beissinger 1999). This hypothesis also proposes that high ambient temperatures cause higher mortality in larger clutches than in smaller clutches, and predicts selection against large clutch sizes, due to the benefits of

reducing the time earlier laid eggs are exposed to high temperatures (Stoleson and Beissinger 1999).

For small passerines, including the House Wren, the estimated normal incubation temperature is between 36°C and 40.5°C (Webb 1987). Eggs exposed to temperatures below physiological zero (~24 - 26°C) will not begin to develop and can remain viable for extended periods (Webb 1987), whereas between 24°C and 36°C, passerine eggs may experience unsynchronized tissue growth and abnormal development, potentially leading to mortality (Stoleson and Beissinger 1999). The length of exposure to intermediate temperatures that are sufficient to kill embryos varies among species; experiments on Green-Rumped Parrotlet (*Forpus passerines*) and House Sparrow (*Passer domesticus*) eggs suggest that three days may be the minimum (Viega 1992, Stoleson and Beissinger 1999). First laid eggs in a clutch will typically have longer exposure to ambient temperatures, and thus should be more susceptible to detrimental effects of temperature.

Support for the egg-viability hypothesis has been equivocal in previous studies. The probability of early onset increases in southern latitudes and later in the season in Eastern Bluebirds (*Sialia sialis*) and Red-winged Blackbirds (*Agelaius phoeniceus*) (Cooper et al. 2005), and with increases in the proportion of daily temperatures above 26°C during the egg laying stage in Tree Swallows (*Tachycineta bicolor*) (Ardia et al. 2006). However, onset is not accelerated by ambient temperatures above physiological zero in several passerine species (Wang and Beissinger 2009). In some, but not all species, females initiate incubation earlier within larger clutches (Magrath 1992, Ardia and Clotfelter 2007, Wang and Beissinger 2009). The per-egg probability of hatching failure is highest for larger clutches at low latitudes and later in the season, and hatching failure does increase over the course of the season in Eastern Bluebirds (Cooper et al. 2005, 2006). While some researchers have found that viability of first-

laid eggs is lower during high ambient temperatures when incubation is delayed (Viñuela 2000), in other cases laying order is not related to egg hatchability at all (Saino et al. 2004).

Physiological evidence suggests that both extremes of hot and cold temperatures shape the complex relationship between incubation behavior and clutch size. Webb (1987) estimated an egg's thermal tolerance for short exposures at 16 to 41°C; to "maintain viability", the poultry industry typically stores eggs between 15 and 20°C (Fasenko 2007). Prior to onset, eggs are thought to have a relatively high tolerance to hypothermia (Weinrich and Baker 1978, Ewert 1991), but the extent to which avian embryos from wild species can tolerate hypothermia is poorly documented (Sockman and Schwabl 1998), and very few studies have examined how hatchability in wild bird eggs varies with exposure to pre-incubation colder temperatures in temperate climates (Webb 1987).

It is important to consider the egg-hypothermia hypothesis, which predicts that females will initiate incubation early to protect the earliest-laid eggs from hypothermia and loss of viability at colder temperatures. This hypothesis also takes into consideration the possibility that large clutches have advantages in cooler weather due to thermal inertia and slower cooling during female off-bouts, based on the clutch cooling hypothesis of Reid et al. (2000). Egg viability is predicted to decrease as the duration of exposure to pre-incubation cold temperatures increases and with increased clutch size. Previous research on wild birds has examined onset, clutch size, and hatching failure in relation to minimum pre-incubation temperature, but rarely in relation to the cumulative amount of time eggs are exposed to cold temperatures (Wang and Beissinger 2009).

To test these two non-mutually exclusive hypotheses in the House Wren (*Troglodytes aedon*), I measured the thermal conditions preceding onset of incubation,

determined when females initiated incubation, and collected data on clutch size and hatching failure. I tested predictions of the egg-viability hypothesis, specifically that females would initiate incubation early and lay smaller clutches when pre-incubation ambient temperatures were above physiological zero (≥ 24°C), and early-laid eggs would fail to hatch more often than later-laid eggs. I contrasted this with the egg-hypothermia hypothesis, which predicted that females would initiate incubation earlier and lay larger clutches when exposed to an increased duration of colder ambient temperatures (≤ 16°C), and that longer cold pre-incubation exposures would result in increased hatching failure, particularly for early-laid eggs.

METHODS

Study Population and Data Collection

House Wrens are small (10 - 12 g), altricial, migratory, secondary cavity nesting passerines, which nest readily in nest boxes, and are relatively tolerant of disturbance (Harper et al. 1992). I chose to study laying and incubation behavior in relation to ambient temperature in this species because only females incubate, they are known to have variable onset of incubation (Harper et al. 1994), clutches vary from three to eight eggs, and incubating females experience a relatively wide range of ambient temperatures throughout the breeding season. In New York, House Wrens are generally double brooded, initiating first broods in mid-May, and second broods in late June or early July. Females rely mostly on exogenous resources during incubation, and male incubation feeding is rare or nonexistent (Johnson and Kermott 1992).

I conducted my research between April and August 2008 at the Cornell University Research Ponds Facility in Ithaca, NY (42.504 N, 76.438 W) where House

Wrens have been studied since 2002. Monitoring was the joint effort of three researchers, each of whom was working on different questions with the House Wren system. The 113 top or front opening nest boxes were mounted on metal poles and distributed over 41 hectares of woodland, interspersed with grassy and marshy areas. Male House Wrens returned to the site in late April; we monitored all nest boxes every three to four days, beginning on 23 April. The males placed one to several sticks within one or more boxes, then a female would line the stick nest before laying eggs. We checked lined nests daily. Once an egg was laid, if I-buttons were inserted (see below), we continued to check the box every day until day six of the nestling period (hatching date of the first chick was "day 1"). The boxes were then periodically checked at least every four days, then every day when fledging approached, in order to determine exact date of fledging.

On the day each egg was laid, we numbered eggs sequentially with an extra-fine-tip permanent marker, writing on the blunt end of the egg. On subsequent days each egg's fate was noted as hatched, disappeared, depredated, or unhatched (failed to hatch). Hatching failure was measured in two ways; first, if hatching failure of one or more eggs occurred (yes or no), and second, as the proportion of each clutch which failed (0 - 0.33). Exact dates of clutch initiation (n = 72 total nests), hatching (n = 51), and fledging (n = 46) were determined. "Early clutches" were nests with a clutch initiation date before the median clutch initiation date of the year (16 June; n = 40), and "late clutches" were those with a clutch initiation date after the median clutch initiation date of the year (n = 32) (Dobbs et al. 2006).

We trapped all adults in mist nets or inside the nest box, weighed each adult to the nearest 0.25 g (30 g spring scale) and measured the tarsus to the nearest 0.1 mm (SPI 150 mm dial plastic caliper). We banded all adults with a U.S. Fish and Wildlife Service aluminum band and three colored leg bands in order to identify individuals,

and to determine if each individual was in a monogamous pair or polygynous trio. All females were captured between the second and thirteenth day hatchings were in the nest; 89% of the females were trapped between the fifth and ninth day.

Onset of Incubation

I determined the onset of diurnal incubation by examining temperature fluctuations over time (representing the females' on and off bouts), measured with I-button data loggers (DS1921G Thermochron iButton, Maxim, Dallas, Texas, USA, 512 bytes of memory; accuracy ± 1°C). On the first day an egg was laid, I placed one I-button in the bottom of the nest cup, adjacent to the eggs, and one I-button on the inner top of the nest box, to measure thermal microclimate in each location (n = 48 nests). I-buttons were set to record temperature to the nearest 0.5°C every one minute and were replaced each day during daylight hours. I downloaded the measurements onto a computer via a DS1402D-DR8 Blue Dot receptor, which attached to the computer via a USB connection. I determined initiation, termination, and length of each on-bout and off-bout by measuring nest cup temperature data. I converted temperature readings from the data loggers into a text file, then converted it into a sound file using Rhythm software (Cooper and Mills 2005). Using Rhythm and Raven software (versions 1.1 and 1.3, respectively; Cornell Laboratory of Ornithology, Ithaca, N.Y., U.S.A.), I developed an algorithm to detect off-bouts as intervals in which temperatures decreased monotonically for at least one minute, dropping at least 1°C, with a minimum initial slope of 0.5°C per min. To ensure accuracy, I visually examined the output for each on- and off-bout to ensure differences in temperature were not a result of the concurrently recorded ambient temperature. In some nests, the nocturnal nest cup temperature fell slowly as the ambient temperature declined, but the

nest cup temperature remained well above the ambient temperature; these intervals were considered on-bouts (Clotfelter and Yasukawa 1999, Wang and Beissinger 2009). Diurnal and nocturnal bouts were assigned according to the civil sunrise and civil twilight for each date at the study site's latitude and longitude (U.S. Naval Observatory, http://aa.usno.navy.mil/cgi-bin/aa_rstablew.pl).

I calculated onset of incubation in two ways: (1) in relation to the day of clutch completion (assigned "0"), to indicate the number of days prior to clutch completion that onset occurred, and (2) in relation to the day the first egg was laid (clutch initiation date assigned "1"). The first number allows for comparison across differing clutch sizes. The second number is related to clutch size and indicates the number of days the first laid egg was exposed to ambient temperatures. Maximum length (in days) of exposure to ambient temperature was calculated for each nest and for each egg. I also categorically labeled onset of incubation for each nest as "early" (onset occurred prior to clutch completion) or "not early" (onset occurred on the day the last egg was laid).

To calculate onset of full incubation, I first determined the number of minutes each female was on the nest each day during laying, by summing the total length of each diurnal off-bout, and subtracting this number from the total number of minutes between civil sunrise and civil twilight. I was unable to determine the number of minutes on the nest for certain days due to the I-button malfunctioning or because the House Wren removed the I-button from the nest. If I-button data was missing for the entire day, and the day was at least two days before or one day after initiation of incubation, the missing data did not impact determination of onset; however, if data was missing on the day prior to the first known day on which incubation occurred, I was not able to determine exact day of onset, as it could have occurred on the day with the missing data. For days on which I calculated the female's presence for part of the

day, I extrapolated to estimate the number of minutes she was on the nest for the total day.

In 43 nests, I counted the number of minutes each female was on the nest throughout all or a portion of "conventional incubation," or the day the last egg was laid through the day before the first egg hatched (n = 113 days of conventional incubation). The minimum number of minutes the female was on the nest during conventional incubation was 258 minutes; therefore, I determined the onset of full incubation for each nest as the day that the female was on the nest for 258 or more minutes. To verify the 258 minute threshold, I plotted the number of minutes each female was on the nest each day against the day of laying in order to verify that there was a sharp rise in the number of minutes on the nest on the day for which onset was determined with the 258 minute threshold.

Environmental Conditions

Ambient temperature was recorded every minute with I-buttons placed in the shade, on the outside bottom of at least two nest boxes at the site. Prior to 10 May, and when there were I-button malfunctions (n = 10 days of the laying period), I used hourly temperature readings from the Ithaca Game Farm Road logger (permanently located approximately 6 km from the study site (http://www.nrcc.cornell.edu/climate/ithaca/gfr_logger.html)). Temperature data are considered more conservative for inferring incubation than visual observation of birds entering the nest box (Wang and Beissinger 2009), and in this study I validated the use of I-buttons to assess off-bouts with visual observations.

I calculated total time the diurnal pre-incubation temperatures were 24°C or above, to indicate how long the first laid egg was exposed to ambient temperatures

above physiological zero (Beissinger et al. 2005), and 16°C or below, to indicate how long the first laid egg was exposed to cold ambient temperatures which may decrease egg viability due to hypothermia (Webb 1987). I also calculated the time each individual egg was exposed to pre-incubation temperatures 24°C or above, and 16°C or below. I assumed each egg was laid before 500 EST.

I used cumulative number of minutes of exposure to high (≥ 24°C) and low (≤ 16°C) temperatures, rather than maximum and minimum temperature, because I assumed that the length of exposure to temperatures which potentially caused hyperthermia or hypothermia would have more of an effect on egg viability than a potentially short exposure to the highest or lowest temperature during the pre-incubation period. For example, the egg-viability hypothesis predicts that exposure to temperatures greater than physiological zero increase mortality due to partial and potentially abnormal development of the egg (Stoleson and Beissinger 1999). I therefore assume that the longer the egg experiences these high temperatures, the greater the potential for abnormal development to occur.

Daily rain (inches) was monitored locally at the Ithaca Game Farm Road logger. Total precipitation relates to adverse foraging conditions (Wang and Beissinger 2009). Weather data were summarized for each nest attempt between 500 and 2100 each day (16 hour interval), starting with the morning of clutch initiation.

STATISTICAL METHODS

I used the statistical program R v. 2.8.0 (R Development Core Team 2008) for all analyses. I tested the response variables for assumptions of normality using Shapiro-Wilk's W. For nine females, I determined onset of incubation for two nests; I avoided pseudoreplication by including only one nest in analyses involving pre-

incubation temperatures, the first nest per female in which I determined onset of incubation (n = 30 nests with exact day of onset determined; n = 31 nests with early onset (yes or no) determined).

In the general linear models (GLMs) and general linear mixed models (GLMMs), I removed in a stepwise backward fashion all independent factors that were not statistically significant at $P > 0.05$. Because variables with $P > 0.05$ were removed from models, I was unable to generate exact P-values for insignificant variables. Final reported values from GLMs and GLMMs consisted of the original response variable and the significant ($P < 0.05$) predictor variables only. All secondary interaction terms were included in each analysis: interactions terms could not all be included at once due to overparameterization as a result of small sample sizes and large number of predictor variables; therefore, interaction terms were included in a random order one or two at a time. No interaction terms were significant, and therefore will not be further discussed.

I used a GLM to determine if nestling age on date captured, time of day captured, Julian date of capture, and the quadratic term of each varied significantly with female weight, in order to include the significant variables in my measure of female body condition. I used GLMs to examine the relationships between individual and environmental predictor variables and early onset of incubation, clutch size and hatching failure, and GLMMs to evaluate which individual and environmental predictor variables varied significantly with per-egg hatching failure, and to determine the probability that an egg with a specific hatching order (first through seventh) was more likely to hatch or not hatch, as compared to eggs with a different hatching order. Clutch size was standardized into normal deviates from the yearly mean (relative clutch size). Female condition, season, breeding status (monogamous versus polygynous) and relative clutch size were included in several analyses to control for

variation attributable to these factors. In each GLM or GLMM for which I included correlated results, I ran the model separately with and without each of the correlated variables; in each model, inclusion or exclusion of correlated variables did not alter which variables were statistically significant. Exclusion of outliers did not alter the significance of the results.

I used a Welch Two Sample t-test to validate that there were no systematic differences between the temperature measured by the Ithaca Game Farm Road logger and the I-buttons at my study site, and a Chi-square test to compare off-bouts determined by I-buttons with visual observations (number of times I-buttons and visual observations both recorded an off-bout, versus number of times an off-bout was recorded by only one method). I used a Wilcoxon rank sum test to determine if the clutch size and likelihood of early onset was significantly different between early and late season nests, a Wilcoxon rank sum test to determine if the mean number of days of exposure to ambient temperature was significantly different between hatched and unhatched eggs, and binomial tests to determine if exposure to at least one day, or at least three days of ambient temperature was associated with an increased probability of an individual egg not hatching. I used a sign test to determine if clutch size and onset of incubation differed between the first and second nest of each female.

RESULTS

Weather Conditions

In the 30 nests used in all the analyses which included temperatures prior to incubation as predictor variables, eggs from one or more nests were exposed to ambient temperatures prior to incubation for a total of 64 different days. During these

64 days, temperatures were hypothermic (≤ 16°C) and above physiological zero (≥ 24°C) on 66% and 73% of the days, respectively (mean = 270 ± SE 33 minutes/day ≤ 16°C; mean = 279 ± SE 39 minutes/day ≥ 24°C). Twenty-eight of the 30 nests (93%) were exposed to diurnal temperatures ≤ 16°C, and 26 of the 30 nests (87%) were exposed to diurnal temperatures ≥ 24°C, for some portion of the pre-incubation laying period. Early season nests were exposed to diurnal temperatures ranging from 0.5 – 34.2°C (mean 15.6 ± SE 0.4°C), and late season nests were exposed to temperatures ranging from 8.1 – 30.9°C (mean 20.1 ± SE 0.2°C).

There were no systematic differences between the temperature measured by the Ithaca Game Farm Road logger and the I-buttons at my study site when both were in operation (n = 35 pairs of hourly readings; $t = 0.351$, $df = 68$, $p = 0.726$).

Female Body Condition

For the 38 females captured from my study area in 2008, female mass did increase significantly with tarsus ($\beta = 0.61 \pm$ SE 0.16, $p < 0.001$) and the quadratic term for nestling age on day of capture ($\beta = -0.01 \pm$ SE 0.002, $p = 0.017$), but did not vary significantly with time of day captured, Julian date of capture, or the quadratic terms of each (P's > 0.05). I therefore estimated relative female body condition as the residuals of body mass regressed against tarsus length and the quadratic term for nestling age on the day the female was captured, to correct for body size and nestling age effects.

Validation of Determination of Onset of Incubation

I validated the use of I-buttons to assess off-bouts with visual observations. These comparisons resulted in a concordance of 95% (n = 61 independent tests) with no significant difference between the observed and I-button assessments (Chi-square test, $X^2 = 0.076$, df = 1, p = 0.783).

Onset of Incubation

I collected I-button temperature data from 43 nests in which nest failure did not occur during laying. In three nests, the data logger malfunctioned, or the female displaced the I-button, and I was not able to determine if early onset occurred. In the remaining nests, I determined if early onset occurred (yes or no) in 40 nests, and exact day of onset for 37 nests. Females initiated incubation prior to clutch completion in 80% of the nests (n = 40). In the first nest per female, early onset of incubation ranged from one to three days prior to clutch completion (n = 30; mean = 1.3 ± SE 0.1, median = 1), and two to eight days from clutch initiation (n = 30; mean = 5.1 ± SE 0.3, median = 5).

Early onset of incubation and temperature

I examined predictions of the egg-hypothermia hypothesis and the extent to which females were constrained in their ability to respond to cold temperatures by initiating incubation early (Table 1). The onset of full incubation was influenced by cold temperatures when they occurred: the probability of a female initiating incubation

Table 1. Predictions of the egg-viability and egg-hypothermia hypotheses.

Hypothesis	Predictions	Prediction supported by results?
Egg-viability	Increased exposure to temperatures ≥ 24°C is associated with early onset of incubation	NO
	Increased exposure to temperatures ≥ 24°C is associated with small clutches	NO
	Large clutches are associated with early onset	NO
	Increased exposure to temperatures ≥ 24°C is associated with increased hatching failure	NO
	Large clutches are associated with increased partial hatching failure of a clutch	NO
	Hatching order significantly predicts likelihood of hatching failure	NO
Egg-Hypothermia	Increased exposure to hypothermic (≤ 16°C) temperatures is associated with early onset of incubation	NO
	Increased exposure to hypothermic (≤ 16°C) temperatures is associated with large clutches	NO
	Large clutches are associated with early onset	NO
	Increased exposure to hypothermic (≤ 16°C) temperatures is associated with increased hatching failure	**YES**
	Hatching order significantly predicts likelihood of hatching failure	NO

*Physiological zero is represented by 24°C.
*"Temperatures" in table refers to pre-incubation temperatures during laying.

early decreased with increases in the cumulative number of minutes the pre-incubation temperatures were hypothermic (≤ 16°C) (n = 30, β = -0.001 ± SE 0.0004, p = 0.004) (Figure 1a). In other words, the longer clutches were exposed to pre-incubation temperatures 16°C or less, the less likely a female was to initiate incubation before the last day an egg was laid. When onset of incubation was measured as days from clutch initiation day (which takes clutch size into account) the result was the same; the longer temperatures were hypothermic (≤ 16°C), the later in the laying period females initiated incubation (n = 30, β = 0.0003 ± SE 0.0001, p = 0.011, Table 2). Thus the longer conditions were unfavorably cold, the greater the exposure of eggs to potentially detrimental ambient temperatures. The probability of a female initiating incubation early did not increase significantly with increasing pre-incubation precipitation levels (Tables 1 and 2).

I then examined predictions of the egg-viability hypothesis, to test if the probability that a female initiated incubation early increased with the cumulative number of minutes the temperatures were above physiological zero (≥ 24°C) (Table 1). Females did not initiate incubation earlier with greater number of minutes pre-incubation temperatures were ≥ 24°C (n = 30; Figure 1b; Table 2). However, early onset was significantly more likely to occur in the late season (after the median egg date) as compared to the early season (before the median egg date) (early onset, early season, n = 10; early onset, late season, n = 13; no early onset, early season, n = 8; no early onset, late season, n = 0; Wilcoxon rank sum test; W = 65, p = 0.007).

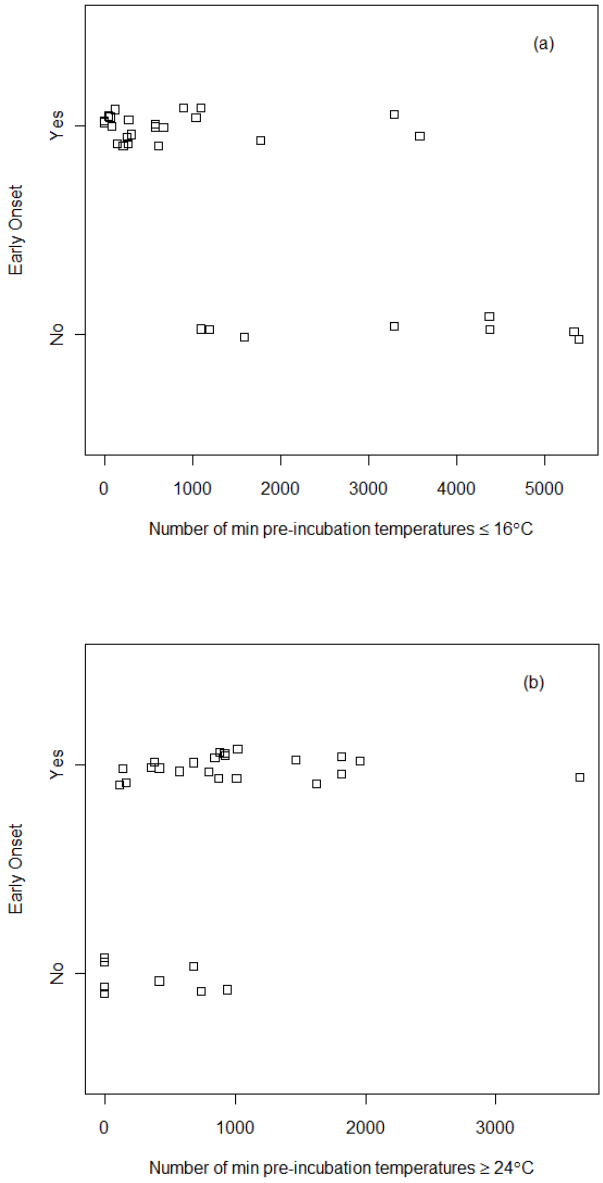

Figure 1. The relationship between early onset of incubation and temperature. (a) Early onset was significantly less likely to occur the longer temperatures were hypothermic (≤ 16°C). **(b)** Early onset was not significantly more likely to occur with increasing cumulative temperatures greater than physiological zero (≥ 24°C).

Table 2. GLM analyses of factors predicting early onset, clutch size, partial hatching failure, and proportion of eggs which failed within clutches.

Model #	**Predictor Variables**								
Response Variables	Female condition	Relative clutch size	Number min ≥24°C	Number min ≤16°C	Total precipitation	Season (early or late)	Early onset (yes or no)	Clutch initiation date	Breeding Status
1. Early onset (y/n)	NS	NS	NS	**p=0.004**	NS	NS	-	-	NS
2. Days after clutch initiation onset occurred	NS	NS	NS	**p=0.011**	NS	NS	-	-	-
3. Clutch size	-	-	-	-	-	-	-	**p < 0.001**	-
4. Clutch size	NS	-	NS	NS	-	NS		-	NS
5. Partial hatching failure (y/n)	NS	NS	NS	**p=0.017**	-	-	NS	-	NS
6. Proportion of clutch failed	NS	NS	NS	**p=0.006**	-	-	NS	-	-

Each dependent variable was analyzed separately and included the covariates listed in the table above. Models 1, 5, and 6 specified a binomial distribution with a logit link, model 2 specified a Gaussian distribution with an identity link, and models 3 and 4, specified a Poisson family with a log link. N = 30 for all analyses. Significant predictor variables in each model are shown with the P-values; β, SE and P-values are included within the text. Nonsignificant predictor variables are labeled "NS"; for all nonsignificant variables, $p > 0.05$. See text for further details.

*Though the following variables were correlated: models 1, 2, 4, 5, and 6 included both the min ≥ 24°C and ≤ 16°C to evaluate effects of higher versus lower temperatures (Spearman's rank, rho = -0.62, $p < 0.001$); models 1, 2 and 4 included both season and the min ≤ 16°C to evaluate effects of seasonal differences versus low temperatures (Spearman's rank, rho = -0.73, $p < 0.001$); and models 4 and 6 included the number of min temperatures were ≤ 16°C and early onset (yes or no) to evaluate both the effects on onset and low temperatures (Spearman's rank, rho = -0.67, $p < 0.001$).

Early onset of incubation, temperature and clutch size

Clutches were initiated between 8 May and 25 July. Excluding the nests in which eggs were abandoned (n = 2) or depredated (n = 3) during laying, clutch sizes ranged from three to eight eggs (n = 67 nests). The mean annual clutch size was 5.9 ± SE 0.1 eggs; the mean size of early and late season clutches were 6.4 ± SE 0.1 (n = 37; mode = 6) and 5.3 ± SE 0.2 eggs (n = 30; mode = 5), respectively.

I examined the predictions of the egg-hypothermia and egg-viability hypotheses, to test if females were more likely to initiate incubation early in larger clutches, and to test if increased exposure during laying to hypothermic (≤ 16°C) temperatures is associated with large clutches, or exposure to temperatures above physiological zero (≥ 24°C) is associated with small clutches (Table 1). The probability of a female initiating full incubation before clutch completion did not increase significantly with clutch size (n = 30; Table 2). Clutch size did not increase with cumulative number of minutes the temperatures were hypothermic (≤ 16°C), nor did it decrease with cumulative number of minutes the temperatures were above physiological zero (≥ 24°C) (n = 30; Table 2).

The earlier the clutch initiation date, the larger the clutch size (n = 30, β = -0.036 ± SE 0.007, $p < 0.001$). In discrete analyses, early season clutches were also significantly larger than late season clutches (early: n = 18, mean 6.5 ± SE 0.1; late: n = 13, mean 5 ± SE 0.4; Wilcoxon rank sum test; W = 196, $p < 0.001$), and the second nests of ten females in which two clutches were monitored was significantly smaller than the first nest of each female (mean clutch size first nest = 6.2 ± SE 0.2, second nest = 5.4 ± SE 0.2; t = 2.522, df = 17, p = 0.022).

Comparison of early onset in females with two clutches

I tested if females were capable of altering their clutch size within the breeding season, and if females would be more likely to initiate incubation early later in the season, when temperatures were warmer. Comparing early onset (yes or no) in the first and second clutch of nine females, three females (30%) initiated incubation before the last day of laying in their second nests only, and six females (60%) initiated incubation early in both their first and second nests. In a comparison of first and second clutches from the same female, the female was significantly more likely to initiate incubation early in second than first clutches (Sign test, $n = 9$, $p = 0.004$).

Hatching Failure

Of the 408 eggs laid in 72 nests in 2008, 62% of the eggs hatched, 11% failed to hatch in the nest after at least one egg had hatched, 7% were broken or missing before the first egg hatched (depredation events including attempted take-overs by House Wrens), and 20% were in nests in which no eggs hatched (depredation or abandonment). At least one egg hatched in 78% of the nests ($n = 72$). Among these clutches in which at least one egg hatched, 50% ($n = 56$) contained one or more unhatched eggs.

Validation that methods did not impact hatching failure

To determine if my field methods influenced hatching failure, I compared the nests in my study area (Unit 2), with nests in a second House Wren study site located 3 km away (Unit 1), which has similar habitat, and in which nests were checked every

three to four days, but eggs were not weighed or labeled and I-buttons were not used. I used a Wilcoxon rank sum test to compare the proportion of unhatched eggs from nests in which at least one egg hatched and in which no eggs were lost or broken prior to hatching. The proportions of unhatched eggs between my study area and Unit 1 were not statistically different (n = 33 and n = 23 nests, respectively; mean proportion unhatched eggs per nest in both Unit 1 and 2 = 0.11 ± SE 0.03; W = 403, p = 0.667), suggesting that my methods did not increase the proportion of unhatched eggs.

Hatching Failure within a Clutch

Hatching failure and temperature

To evaluate predictions of the egg-hypothermia and egg-viability hypotheses, I examined if hatching failure was more likely with increased exposure to cold (≤ 16°C) and warm temperatures (≥ 24°C) (Table 1). Hatching failure was influenced by cold temperatures: the greater the cumulative number of minutes the temperatures were hypothermic (≤ 16°C), the higher the probability of partial hatching failure (n = 30, β = 0.001 ± SE 0.0004, p = 0.017), and the higher the proportion of eggs failing per clutch (n = 30, β = 0.0003 ± SE 0.0001, p = 0.006) (Figure 2a; Table 2). Neither partial hatching failure (yes or no), nor proportion of eggs failing per clutch, increased with the cumulative number of minutes the temperatures were above physiological zero (≥ 24°C) (n = 30; Figure 2b; Table 2).

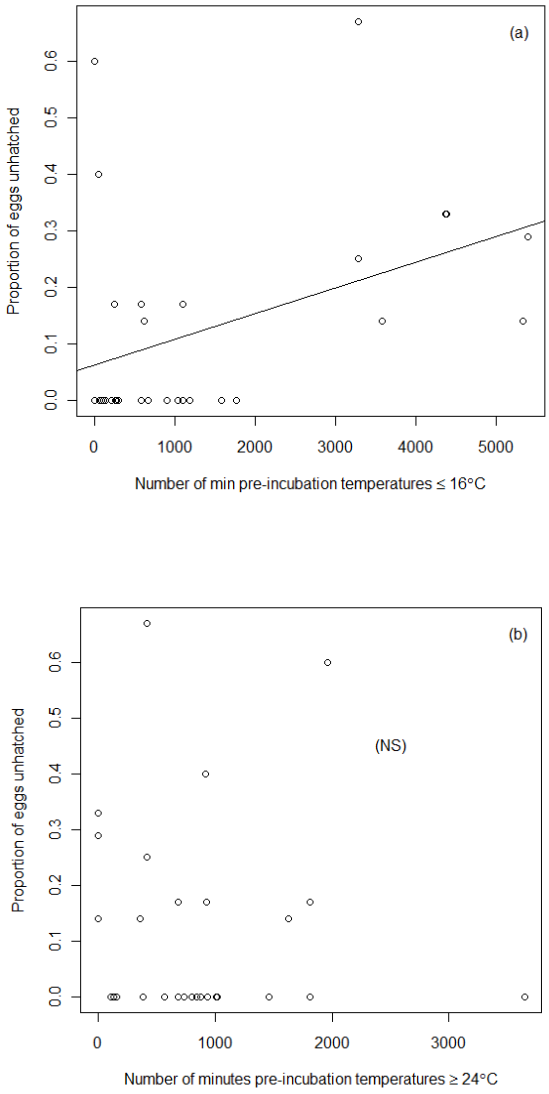

Figure 2. The relationship between proportion of unhatched eggs and temperature. (a) Hatching failure was influenced by cold temperatures: a statistically significantly higher proportion of eggs within a clutch failed to hatch with increasing cumulative number of min the temperatures were hypothermic (≤ 16° C). **(b)** Hatching failure was not influenced by temperatures above physiological zero: the proportion of eggs failing within a clutch did not increase with increasing cumulative time temperatures were above physiological zero (≥ 24°C).

Hatching failure and clutch size

I examined the egg-hypothermia and egg-viability hypotheses' prediction that the probability of hatching failure would increase in larger clutches (Table 1). I used a larger data set (n = 39 nests) to evaluate if hatching failure was associated with clutch size. Partial hatching failure (yes or no) tended to be more likely in larger clutches, but the difference was not statistically significant ($\beta = 0.800 \pm$ SE 0.430, $p = 0.063$). The proportion of failed eggs in the nest also did not vary significantly with clutch size ($\beta = -0.070 \pm$ SE 0.209, $p = 0.739$).

Hatching failure and onset of incubation

I then evaluated if hatching failure was less likely when early onset of incubation occurred, as would be predicted by the egg-hypothermia and egg-viability hypotheses (Table 1). In 31 nests, neither partial hatching failure (yes or no), nor proportion of eggs that failed per clutch decreased when early onset of incubation occurred (Figure 3; Table 2).

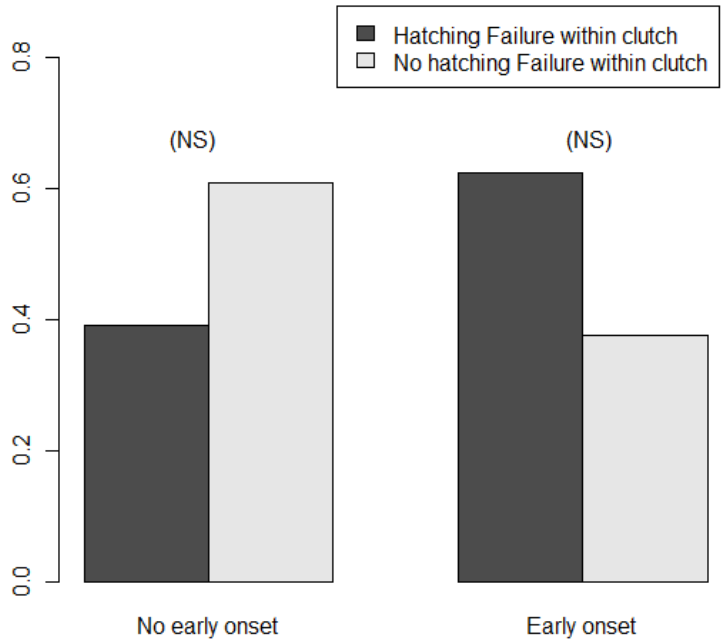

Figure 3. The relationship between hatching failure and early onset. The proportion of eggs that hatched per clutch did not increase with early onset.

Per-Egg Hatching Failure

Per-egg hatching failure and temperature

If exposure to ambient temperatures increases the probability of hatching failure, I would predict that the greater the number of days an egg is laid prior to onset of incubation, the higher the likelihood of hatching failure. If an egg was laid prior to onset of incubation, it was exposed to at least one full day of ambient temperature. In 30 nests for which onset day and hatch order were known, 65% of 160 eggs were laid

before the onset of incubation; 71% of the 21 eggs that failed to hatch (n = 12 nests) were laid before the onset of incubation. Eggs exposed to at least one day or a minimum of three days of ambient temperature did not have a reduced probability of hatching (out of 139 hatched and 21 unhatched eggs, n = 89 and n = 15 exposed to at least 1 day of exposure, n = 45 and n = 9 exposed to at least 3 days of exposure, respectively; Binomial test, $X^2 = 0.174$, df = 1, p = 0.677; $X^2 = 0.489$, df = 1, p = 0.484, respectively). Hatched eggs tended to be exposed to fewer days of ambient temperature compared to unhatched eggs, but the difference was not statistically different (1.7 ± SE 0.1 *v.* 2.3 ± SE 0.5 , $n_1 = 139$ eggs, $n_2 = 21$ eggs; Wilcoxon rank sum test, W = 170, p = 0.205).

The egg hypothermia hypothesis predicts that per-egg hatching failure will be influenced by cold temperatures; whereas the egg-viability hypothesis predicts the per-egg hatching failure will be influenced by warm temperatures. The probability of an egg not hatching increased with the cumulative number of minutes the egg was exposed to hypothermic (≤ 16°C) temperatures (n = 200 eggs, β = -0.0004 ± SE 0.0002, $t = -2.293_{37}$, p = 0.022, Table 3). The probability of an egg not hatching did not increase with the cumulative number of minutes the egg was exposed to temperatures above physiological zero (≥ 24°C) (Table 1).

Per-egg hatching failure and hatching order

The egg-hypothermia and egg-viability hypotheses predict that hatching order significantly predicts likelihood of hatching failure, and that earlier laid eggs are more likely to fail than late laid eggs, as earlier laid eggs are more likely to be exposed to ambient temperatures (Table 1). In 37 nests (first nests per female, both nests in which

Table 3. GLMM analyses of factors predicting the likelihood of an individual egg hatching and per-egg hatching failure.

Model #	**Fixed Predictor Variables**					**Random Effect**
Response Variables	Hatching Order (1-7)	Female condition	Relative clutch size	Number min ≥24°C	Number min ≤16°C	Nest Number
1. Egg Hatched (y/n)	NS	-	-	-	-	√
2. Egg Hatched (y/n)	NS	-	-	-	-	√
3. Per-egg hatching failure	NS	NS	NS	NS	**p = 0.022**	√

Each dependent variable was analyzed separately and included the covariates listed in the table above. Models 1, 2 and 3 specified a binomial distribution with a logit link. In models 1 and 3, I analyzed eggs from the first nest per female in which at least one egg of known hatching order was known to have hatched or not (n = 37 nests; n = 200 eggs of known hatching order and known fate (hatched/unhatched)). In model 2, I analyzed eggs from the first nest per female in which at least one egg of known hatching order was known to have hatched or not, and eggs experienced at least one day of exposure to ambient temperatures (n = 27 nests; n = 105 eggs). Significant predictor variables in each model are shown with the P-values; β, SE and P-values are included within the text. Nonsignificant predictor variables are labeled "NS"; for all nonsignificant variables, $p > 0.05$. The predictor variables, number min ≥ 24°C and ≤ 16°C, are calculated for the time period during laying, prior to onset. See text for further details.

*Model 3 included both the number of min ≤ 16°C and ≥ 24°C even though the variables were significantly correlated, in order to account for effects of low versus high temperatures (Spearman's rank, rho = 0.22, p = 0.006).

onset was and in which onset was not determined), for 200 eggs of known laying order and fate, the hatching order (egg hatched first through seventh) did not have any significant effect on hatching probability ($\beta = 0.114 \pm$ SE 0.135, $t = 0.843_{37}$, $p = 0.399$, Table 3). A total of 105 eggs laid prior to onset of incubation were of known hatch order and fate. Of these eggs, which were exposed to at least one day of ambient temperature, the hatching order did not have any significant effect on hatching probability ($\beta = 0.015 \pm$ SE 0.093, $t = 0.165_{27}$, $p = 0.870$). Clutch size was included as a covariate, and controlling for clutch size did not alter the significance of the results. In order to determine whether hatchability increased with clutch size, as would be expected if females in better condition tend to lay larger clutches, I evaluated if the first eggs laid in 39 first nests of females, were less likely to hatch in smaller clutches. The likelihood of the first egg hatching did not decrease in smaller clutches ($\beta = -0.600 \pm$ SE 0.593, $p = 0.312$).

DISCUSSION

I examined the impact of warm and cold temperatures on female House Wrens' laying and incubation behavior to evaluate the degree to which females altered their strategy to protect egg viability. Both high and low ambient temperatures may impact avian egg viability, but my results suggest that in my study population in Ithaca, New York, only exposure to hypothermic (≤ 16°C), not warm (≥ 24°C) pre-incubation temperatures is associated with hatching failure. Temperatures below and above these thresholds were common on my study site: the temperatures during the 2008 House Wren breeding season were hypothermic (≤ 16°C) 66% and above physiological zero (≥ 24°C) 73% of the 64 days on which eggs were exposed to ambient temperatures. The likelihood of partial hatching failure within a clutch, the

proportion of eggs failing per clutch, and the per-egg probability of not hatching all increased with the cumulative time pre-incubation temperatures were hypothermic (≤ 16°C). As these same measures did not increase with pre-incubation temperatures 24°C or above, my results suggest that patterns of hatching failure are consistent with the egg-hypothermia hypothesis, and do not support the egg-viability hypothesis.

Few studies have examined how hatchability in wild bird eggs varies with exposure to pre-incubation cold temperatures in temperate climates (Webb 1987); although eggs are thought to have a relatively high tolerance to hypothermia (Weinrich and Baker 1978) the extent to which avian embryos from wild species can tolerate hypothermia is poorly documented (Sockman and Schwabl 1998). Wang and Beissinger (2009) included minimum temperature as a predictor variable to evaluate effects of cold temperatures on egg viability, but they did not find a significant effect on hatching failure or initiation of incubation. Minimum temperature may be correlated with amount of exposure to cold temperatures, but likely has a different effect on egg viability than cumulative exposure. In my study, I used duration of exposure to colder temperatures (≤ 16°C) as an explanatory variable, in order to evaluate the association between duration of hypothermic temperatures, hatching failure, onset of incubation and clutch size. My results suggest, contrary to common belief (Webb 1987), that in temperate climates, hypothermia sometimes poses a higher risk to egg viability than hyperthermia, based on my observation that hatchability declines with exposure to colder, but not warmer temperatures.

The egg-hypothermia and egg-viability hypotheses predict that females respond to cold and warm temperatures, respectively, by initiating incubation early to prevent a decline in egg viability. The assumption of the egg-hypothermia hypothesis, that eggs exposed longer to hypothermic (≤ 16°C) temperatures will have higher mortality, was supported, but the predicted female behavioral response was not

upheld: females did not initiate incubation earlier when ambient temperatures were cold enough to decrease egg viability (≤ 16°C). Contrary to predictions of the egg-hypothermia hypothesis, the probability of a female initiating full incubation early actually decreased the longer the ambient temperatures were 16°C or below. In other words, when ambient temperatures were colder for longer, females allocated less, rather than more, time to incubation.

Why did females not initiate full incubation prior to clutch completion to counteract the negative effects of cold temperatures on hatching success? This may be explained by the energy constraints hypothesis, which predicts that when ambient temperatures are cold, there are fewer insects, and due to the lower food availability and higher thermoregulatory costs related to the colder weather, females spend more time on self-maintenance, foraging instead of allocating energy to incubation (Nilsson 1993, Nilsson and Svensson 1993). In this study, female House Wrens behaved as if there is a tradeoff between early onset and self-maintenance: the longer temperatures were hypothermic (less food, higher thermoregulatory costs), the less likely females were to initiate full incubation early. In other words, my results suggest that when the birds have enough energy, they initiate early, but when there are adverse environmental conditions (cold weather) they often seem to put self-maintenance before egg viability.

This tradeoff also makes some sense in light of the result that the majority of eggs in each nest tended to hatch, even when some were lost. Nests with at least one hatchling and one or more unhatched eggs contained a median of one unhatched egg and a mean of 1.6 ± SE 0.2 unhatched eggs out of a mean annual clutch size of 5.9 ± SE 0.1 eggs (n = 67 nests). These results suggest that while females reduced their allocation to incubation under cold conditions, most eggs still hatched and relatively few were lost. It is also possible that lower temperatures are associated with less

available food, which led to females being in poor condition. Females in poor condition may have laid lower quality eggs, which were more likely to fail.

In my study population, females did not respond to warmer temperatures (≥ 24°C) by initiating incubation prior to clutch completion. The likelihood of a female House Wren initiating incubation early did not vary significantly with the cumulative time temperatures were 24°C or above. My results agreed with those of Wang and Beissinger (2009), who found that the proportion of time above 24°C did not vary significantly with onset of incubation in several bird species in northern California. The lack of early initiation when temperatures were warm may be explained by my observation that warm temperatures did not decrease egg viability, and therefore, early onset was not necessary to ensure hatching success.

I evaluated whether females with two nests initiated incubation at different times in the laying cycle (before the last egg was laid or on the day the last egg was laid), in order to determine if the variation within the population could be attributed to different females having different life history strategies: either always initiating incubation early, always initiating incubation on the last day an egg is laid, or varying onset systematically between clutches. For nine females with two nests, I found females were statistically significantly more likely to initiate incubation early in their second nest. My results show that females are capable of using different strategies in different clutches during the same season, suggesting that they are responding to individual or environmental cues when initiating incubation. However, it would take a larger sample size to determine with confidence whether females are more prone to initiate incubation early in second than first nests.

Previous research has suggested that females vary clutch size to decrease the negative effects of ambient temperature on egg viability, potentially laying smaller clutches in higher temperatures to decrease exposure of first laid eggs (Cooper et al.

2005). In this study, however, I found no evidence that high ambient temperatures (≥ 24°C) caused mortality. Females laid statistically larger clutches earlier in the season, when spring temperatures were typically colder, and smaller clutches in the late season, when summer temperatures were warmer overall. Therefore, females may be laying larger clutches when the temperatures are on average colder, and smaller clutches when the temperatures are on average warmer. This would balance the effects of temperature, although females did not seem to alter their clutch size as an immediate response to higher or lower temperatures during laying.

My results provide no evidence that larger clutch sizes are associated with early onset of incubation, contrary to predictions of the egg-hypothermia and egg-viability hypotheses and results of previous studies (e.g. Wang and Beissinger 2009). Females were not incubating early to protect eggs in large clutches from longer exposure to ambient temperature. Larger clutch sizes also did not have higher incidence of partial hatching failure, nor did they exhibit higher proportions of unhatched eggs per clutch. Because larger clutches were not significantly more likely to contain unhatched eggs, early onset of incubation does not seem to be beneficial in protecting egg viability. This may be because only hypothermic (≤ 16°C) temperatures, not temperatures above physiological zero (≥24°C), were associated with reduced egg viability; length of exposure (without separating out effects of cold or warm temperatures) did not significantly decrease egg viability, suggesting that it is not length of exposure only, but exposure to specific temperatures which leads to a decrease in egg viability.

Previous research has focused on predictions of the egg-viability hypothesis for explaining onset of incubation and clutch size trends; however, although other researchers have found that temperatures above physiological zero impact incubation patterns, clutch size, and egg viability (Ardia et al. 2009), I found that temperatures

above physiological zero did not seem to influence initiation of onset, clutch size or hatching failure. In agreement with my results, Wang and Beissinger (2009) found that constraints on adult behavior seemed to be more influential than environmental temperatures on the onset of incubation. Wang and Beissinger (2009) however, predicted that onset would be delayed in cold weather because eggs can remain in stasis below physiological zero, a prediction my study did not uphold, as many eggs lost viability in cold ($\leq 16°C$) weather.

The variation in results of previous studies may be due to different environmental and life-history traits of the study populations, such as early onset ranging from one to three days prior to clutch completion and two to eight days from clutch initiation in my population, whereas in Ardia et al.'s (2006) Tree Swallow population, onset typically occurred only on the last egg day or on the day the penultimate egg was laid. Differing results may be due to variation in impact of temperatures on egg viability of different species, or onset and clutch size being more closely related to high temperatures in warmer climates (such as in Stoleson and Beissinger's (1999) Green-Rumped Parrotlet studies in the tropical climate of Venezuela). In temperate climates, warm temperature may have a strong influence on incubation behavior, but this study suggests a need to explore both extremes of temperature and other environmental variables.

Possible benefits of early onset, other than increased egg viability, may be increased hatching asynchrony, which may be beneficial to parents as a mechanism for reducing the brood, though asynchronous hatching may also be costly, as the youngest chick may die (Lack 1954). However, variation in synchrony is actually suggested to be nonadaptive and unrelated to fitness in the House Wrens: in House Wrens, brood reduction does not typically occur in asynchronous nests, and reproductive success, juvenile survival and offspring recruitment of synchronous and asynchronous broods

do not differ (Harper et al. 1992, 1994). In birds such as the House Wren, in which asynchrony due to onset does not have negative consequences, another explanation is needed to resolve the question of why females initiate incubation early when not constrained by cold conditions. Possible explanations that have been put forth include faster development, which might decrease likelihood of predation (Clark and Wilson 1981, Amundsen and Stokland 1988), protection of eggs from brood parasitisism (Lombardo et al. 1989), or protection from intra- or interspecific nest site competitors (Beissinger and Waltman 1991).

These results suggest that onset of incubation is likely related to environmental or individual conditions and is not simply related to ambient temperature through its effect on egg viability. House Wrens seemed to initiate incubation early when the costs of allocating energy to heating eggs were low, but when the tradeoffs were high, during cold weather, the birds did not initiate incubation prior to clutch completion. The findings that exposure to cold weather increases hatching failure, and that females seem unable to absorb the costs of starting incubating early to prevent mortality, suggest that egg exposure to cold weather has more of an impact on hatching success than previously thought.

CONCLUSION

A better understanding of species-specific behavioral and reproductive responses to ambient temperatures will enable us to predict and model responses to global climate change. My study addressed the egg-viability and egg-hypothermia hypotheses to evaluate in a comprehensive way the behavioral responses of female House Wrens to variation in the ambient thermal microclimate of nest sites. Although I did not conduct a manipulative experiment, which reduces the strength of my

inferences, my results suggested that when temperatures are above physiological zero (≥ 24°C), females did not initiate incubation early to protect eggs from exposure to temperatures above physiological zero and apparently did not incur reduced hatching success by failing to do so. However, with exposure to colder temperatures (≤ 16°C), eggs lost viability and females appeared to face a tradeoff between maintenance and reproduction. Decreased egg viability due to hypothermia appeared to be less important than self-maintenance, leading females to postpone incubation until clutch completion, even though viability of some eggs was reduced. These results suggest that temperate species may vary considerably in their responses to ambient temperature, making cross-species inferences with regard to behavioral response to climate change challenging.

REFERENCES

Ahola, M., T. Laaksonen, K. Sippola, T. Eeva, K. Rainio, and E. Lehikoinen. 2004. Variation in climate warming along the migration route uncouples arrival and breeding dates. Global Change Biology 10: 1610–1617.

Ahola, M., T. Laaksonen, T. Eeva, and E. Lehikoinen. 2009. Great tits lay increasingly smaller clutches than selected for: a study of climate- and density-related changes in reproductive traits. Journal of Animal Ecology 78: 1298–1306.

Amundsen, T. and J. N. Stokland. 1988. Adaptive significance of asynchronous hatching in the Shag: a test of the brood reduction hypothesis. Journal of Animal Ecology 57: 329-344.

Ardia, D. R., C. B. Cooper, and A. A. Dhondt. 2006. Warm temperatures lead to early onset of incubation, shorter incubation periods and greater hatching asynchrony in tree swallows *Tachycineta bicolor* at the extremes of their range. Journal of Avian Biology 37 (2): 137–142.

Ardia, D. R., J. H. Pérez, E. K. Chad, M. A. Voss, and E. D. Clotfelter. 2009. Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behavior. Journal of Animal Ecology 78: 4-13.

Ardia, D. R. and E. D. Clotfelter. 2007. Individual quality and age affect responses to an energetic constraint in cavity-nesting bird. Behavioral Ecology 18: 259–266.

Beissinger, S. R., M. I. Cook, and W. J. Arendt. 2005. The shelf life of bird eggs: testing egg viability using a tropical climate gradient. Ecology 86 (8): 2164-2175.

Beissinger, S. R. and J. R. Waltman. 1991. Extraodinary clutch size and hatching asynchrony of a neotropical parrot. Auk 108: 863-871.

Bradshaw, W. E. and C. M. Holzapfel. 2001. Genetic shift in photoperiodic response correlated with global warming. Proceedings of the National Academy of Sciences 98 (25): 14509-14511.

Clark, A. and D. Wilson. 1981. Avian breeding adaptations: hatching asynchrony, brood reduction, and nest failure. The Quarterly Review of Biology 56: 253-277.

Clotfelter, E. D. and K. Yasukawa. 1999. The function of early onset of nocturnal incubation in red-winged blackbirds. The Auk 116 (2): 417-426.

Cooper, C. B., W. M. Hochachka, G. Butcher, and A. A. Dhondt. 2005. Seasonal and latitudinal trends in clutch size: thermal constraints during laying and incubation. Ecology 86 (8): 2018–2031.

Cooper, C. B. and H. Mills. 2005. New software for quantifying incubation behavior from time-series recordings. Journal of Field Ornithology 76: 352–356.

Cooper, C. B., W. M. Hochachka, T. B. Phillips, and A. A. Dhondt. 2006. Geographical and seasonal gradients in hatching failure in eastern bluebirds *Sialia sialis* reinforce clutch size trends. Ibis 148 (2): 221-230.

Dobbs, R. C., J. D. Styrsky, and C. F. Thompson. 2006. Clutch size and the costs of incubation in the house wren. Behavioral Ecology 17 (5): 849-856.

Ewert, M. A. 1991. Cold torpor, diapause, delayed hatching and aestivation in reptiles and birds. Pages 173-192 *in* Egg incubation: its effects on embryonic development in birds and reptiles. (D. C. Deeming and M. W. J. Ferguson, Eds.) Cambridge University Press, Cambridge, UK.

Fasenko, G. M. 2007. Egg storage and the embryo. Poultry Science 86: 1020-1024.

Harper, R. G., S. A. Julianoa, and C. F. Thompon. 1992. Hatching asynchrony in the house wren, *Troglodytes aedon:* a test of the brood-reduction hypothesis. Behavioral Ecology 3: 76-83.

Harper, R. G., S. A. Julianoa, and C. F. Thompon. 1994. Intrapopulation variation in hatching synchrony in house wrens: a test of the individual optimization hypothesis. The Auk 111: 516-524.

Hill, J. K., C. D. Thomas, and B. Huntley. 1999. Climate and habitat availability determine twentieth century changes in a butterfly's range margins. Proceedings of the Royal Society B 266: 1197-1206.

Humphries, M. M., D. W. Thomas, and J. R. Speakman. 2002. Climate-mediated energetic constraints on the distribution of hibernating mammals. Nature 418: 313-316.

Johnson, L. S. and L. H. Kermott. 1992. Why do male house wrens feed their incubating mates so rarely? American Midland Naturalist 127 (1): 200.

Lack, D. 1954. The Natural Regulation of Animal Numbers. Oxford Press, London.

Lombardo, M. P., H. W. Power, P. C. Stouffer, L. C. Romagnano, and A. S. Hoffenberg. 1989. Egg removal and intraspecific brood parasitism in the European starling (*Sturnus vulgaris*). Behavioral Ecology and Sociobiology 24: 217-223.

Magrath, R. D. 1992. Roles of egg mass and incubation pattern in establishment of hatching hierarchies in the blackbird (*Turdus merula*). The Auk 109: 474-487.

Nilsson, J. 1993. Energetic constraints on hatching asynchrony. American Naturalist 141 (1): 19158-19166.

Nilsson, J. and E. Svensson. 1993. Energy constraints and ultimate decisions during egg-laying in the blue tit. Ecology 74 (1): 244-251.

Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

Pendlebury, C. J., M. G. MacLeod, and D.M. Bryant. 2004. Variation in temperature increases the cost of living in birds. Journal of Experimental Biology 207: 2065–2070.

Peñuelas, J., I. Filella, and P. Comas. 2002. Changed plant and animal life cycle from 1952 to 2000 in the Mediterranean region. Global Change Biology 8: 531-544.

Reid, J. M., P. Monaghan, and G. D. Ruxton. 2000. Resource allocation between reproductive phases: the importance of thermal conditions in determining the cost of incubation. Proceedings of the Royal Society London B 267: 37-41.

Saino, N., M. Romano, R. Ambrosini, R. P. Ferrari, and A. P. Møller. 2004. Timing of reproduction and egg quality covary with temperature in the insectivorous barn swallow, *Hirundo rustica.* Functional Ecology 18: 50-57.

Slagsvold, T. and J. T. Lifjeld. 1989. Constraints on hatching asynchrony and egg size in pied flycatchers. Journal of Animal Ecology 58: 837-849.

Sockman, K. W. and H. Schwabl. 1998. Hypothermic tolerance in an embryonic American kestrel (*Falco sparverius*). Canadian Journal of Zoology 76: 1399-1402.

Stoleson, S. H. and S. R. Beissinger. 1995. Hatching asynchrony and the onset of incubation in birds, revisited. When is the critical period? Pages 191-270 *in* Current Ornithology, vol. 12. (M. Power, Ed.). Plenum, New York.

Stoleson, S. H. and S. R. Beissinger. 1999. Egg viability as a constraint on hatching synchrony at high ambient temperatures. Journal of Animal Ecology 68: 951–962.

Viega, J. P. 1992. Hatching asynchrony in the house sparrow: a test of the egg-viability hypothesis. The American Naturalist 139 (3): 669-675.

Viñuela, J. 1999. Sibling aggression, hatching asynchrony, and nestling mortality in the black kite (*Milvus migrans*). Behavioral Ecology and Sociobiology 45 (1): 33-45.

Viñuela, J. 2000. Opposing selective pressures on hatching asynchrony: egg viability, brood reduction, and nestling growth. Behavioral Ecology and Sociobiology 48: 333-343.

Walther, G., S. Berger, and M. T. Sykes. An ecological 'footprint' of climate change. 2005. Proceedings of the Royal Society London B 272: 1427-1432.

Wang, J. M. and S. R. Beissinger. 2009. Variation in the onset of incubation and its influence on avian hatching success and asynchrony. Animal Behaviour 78: 601-613.

Webb, D. R. 1987. Thermal tolerance of avian embryos: a review. The Condor 89 (4): 874-898.

Weinrich, J. A. and J. R. Baker. 1978. Adelie penguin *Pygoscelis adeliae* embryonic development at different temperatures. The Auk 95 (3): 569-576.